Nick Translation (FISH)

Reagents

Biotin-16-dUTP (100mM) (see Notes)
Boehringer Mannheim, Cat. 109 3070
Bovine serum albumin (BSA)
dATP, dTTP, dGTP, dCTP
Boehringer Mannheim, Cat.: 105 1440,105 1458, 105 1466, 105 1482
DNase I from bovine pancreas
Boehringer Mannheim, Cat. 104 159, 100 mg
99.5% Glycerol
Gibco BRL, Cat. 15514-011
Magnesium chloride ($MgCl_2$) (0.5 M)
β-Mercaptoethanol Solution (99%)
Sigma, Cat. M6250
DNA Polymerase (Kornberg)
Boehringer Mannheim, Cat. 104 485, 500 U
NaCl (1 M)
Tris-HCl (1 M), pH 8.0
Quality Biologicals, Cat. 351-007-100
H_2O

Preparation

Bovine DNase stock solution, 1mg/ml
Dnase, 10 mg
NaCl (1M), 1.5 ml to get a final concentration of 0.15 M
Glycerol (100%), 5 ml to get a final concentration of 50%
H_2O to total volume of 10 ml
Mix well and store at –20˚C.

0.1M β–Mercaptoethanol
β−Mercaptoethanol solution (99%/14.4 M), 34.7 µl
H_2O to a total volume of 5 ml
Mix well aliquot and store at –20°C.

dNTP
dATP (100 mM), 5 µl for a final concentration of 0.5 mM
dCTP (100 mM), 5 µl for a final concentration of 0.5 mM
dGTP (100 mM), 5 µl for a final concentration of 0.5 mM
dTTP (100 mM), 1 µl for a final concentration of 0.05 mM
H_2O, 984 µl
Mix well, aliquot, and store at –20°C

10X NT-Buffer
Tris-HCL (1 M, pH 8.0), 500 µl for a final concentration [0.5 M]
$MgCl_2$(0.5 M), 100 µl for a final concentration [50 mM]
BSA (10 mg/ml), 50 µl for a final concentration [0.5 mg/ml]
H_2O, 350 µl
Mix well, aliquot, and store at -20°C.

Procedure

1. Combine components for 100 µl Nick Translation Reaction

DNA	X µl (2 µg)
10X NT Buffer	10 µl
0.1M β-mercaptoethanol	10 µl
dNTP	10 µl
BIO-16-dUTP	4 µl
Sterile H_2O	60 µl – X µl
Diluted Dnase (note)	4 µl
Polymerase (Kornberg)	2 µl

Total volume of 100 µl.
Mix and briefly centrifuge tube.

2. Place tube into a 15°C waterbath for 1 hr for the nick translation reaction.

3. Stop the reaction by placing tube on ice.

4. Run gel with 5 µl of sample and 0.5 ml of loading buffer.

5. Length of DNA should be about 300 bp-600 bp. See image.

Notes

1. Although Biotin is the only analog listed as the label for the DNA, other analogs can be used such as Digoxygenin-11-dUTP (Boehringer Mannheim, Cat. 155 8706)

2. DNase should be diluted 1:1000 for the initial use and adjusted up or down according to the reaction velocity. See the image. If the DNase reaction velocity is too high, it will result in fragments that are too small. See lanes 7,8,9. One should increase the dilution factor (for example 0.8:1000). If the Dnase reaction velocity is too slow it will result in fragments that are too large. See lane 2. One should decrease the dilution (for example 1.2:1000).